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Molecular Cardiovascular Magnetic Resonance: Current Status and Future Prospects

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Abstract

In the Western world and developing countries, the number one causes of mortality and morbidity result from cardiovascular diseases. Cardiovascular diseases represent a wide range of pathologies, including myocardial infarction, peripheral vascular disease, and cerebrovascular disease, which are all linked by a common cause - atherosclerosis. Currently, the diagnosis of atherosclerosis is in most cases established at the end stage of the disease, when patients are administered to the emergency room due to a myocardial infarction or stroke. Even though cardiovascular diseases have an enormous impact on society, there are still limitations in the early diagnosis and the prevention of the disease. Current imaging methods mainly focus on morphological changes that occur at an advanced disease stage, e.g., degree of stenosis. Cardiovascular magnetic resonance imaging and specifically molecular cardiovascular magnetic resonance imaging are capable to reveal pathophysiological changes already occurring during early atherosclerotic plaque formation. This allows for the assessment of cardiovascular disease on a level, which goes beyond morphological or anatomical criteria. In this review, we will introduce promising MR-based molecular imaging strategies for the non-invasive assessment of cardiovascular disease.

Keywords

Molecular magnetic resonance imaging, Cardiovascular MRI, Molecular probes, Atherosclerosis

Introduction

Molecular cardiovascular magnetic resonance imaging (MRI) is a non-invasive technique for the visualization of molecular and cellular processes in vivo using molecular probes. It provides insights into early biological changes occurring during the development of atherosclerosis, visualization of unstable plaques, and the evaluation of response to therapy [1]. The advantages of MRI, which are especially relevant in the context of cardiovascular diseases, are its high temporal/spatial imaging resolution, the high soft tissue contrast, and the ability to display cardiovascular morphology, physiology, and molecular events within one imaging modality [2]. Additionally, MRI does not rely on the use of ionizing radiation.

Collected data from over 190 countries recently showed that cardiovascular diseases, including atherosclerosis of the aorta, carotid arteries, and coronary arteries, continue to be the leading cause of death in industrialized and developing countries [3]. The early detection of unstable or vulnerable atherosclerotic lesions that are at high risk for rupture remains of high clinical interest as various recent and older studies demonstrated that the degree of luminal narrowing of the vessel lumen does not allow the differentiation between stable and unstable plaques [4–6]. The emphasis of conventional invasive and non-invasive imaging techniques such as myocardial perfusion cintigraphy, CT- or X-ray-

angiography is currently on the assessment of compromised blood flow and grading of luminal stenosis, with the latter (two) method(s) being the current gold standard for the diagnosis of clinical significant vascular stenosis. Considering that positive remodelling preserves the diameter of the vessel lumen by compensatory enlargement of the arterial cross section during atherosclerotic plaque development [6] and plaque features such as a high lipid content and macrophage count are currently not visualized in a clinical setting [5], new imaging procedures and strategies for the detection of plaque vulnerability are required.

Molecular cardiovascular magnetic resonance imaging is a promising imaging technique for a reliable characterization of plaque features and biological processes of plaque progression and destabilization [7, 8]. Atherosclerotic plaque components and morphological features of the carotid, aortic, and coronary artery wall can already be evaluated with clinically established MRI sequences [9–11]. Specific molecular MR probes enable the visualization of molecular and cellular processes and can be generally separated into two different classes: [1] T1- shortening molecular probes (mostly based on paramagnetic gadolinium chelates) and [2] T2-shortening molecular probes (mostly based on iron-oxide-nanoparticles). Additionally, molecularMRprobes allow the characterization and visualization of early changes of disease and thus ameliorate therapy planning and monitoring. The aim of this review is to outline the role of MRI in cardiovascular molecular imaging.

Pathophysiology of Atherosclerosis

Atherosclerosis is characterized by slowly evolving intimal lesions of the inner arterial wall that can protrude into the vascular lumina. With increasing size atherosclerotic plaques can gradually occlude lumina and first symptoms may occur resulting from compromised arterial blood flow. Acute clinical symptoms appear in most cases due to atherosclerotic plaque rupture, thrombosis, hemorrhage, or embolization [12, 13]. Chronic or repetitive endothelial injury leads to an inflammatory cascade and results in endothelial dysfunction with increased permeability, accumulation of low density lipoproteins (LDL) into the subendothelial tissue, monocyte adhesion to the endothelium, subsequent migration into the intima, and transformation of monocytes into macrophages and ultimately into cholesterol-accumulating foam cells within the atherosclerotic plaque matrix [12, 14]. The initial accumulation of lipid-containing macrophages in the intima is referred to as Bfatty streaks.^ Further steps comprise platelet activation and factor release from activated platelets, macrophages, and vascular wall cells to induce recruitment of smooth muscle cells [12]. Extracellular matrix (ECM) deposition, e.g. elastin, collagen, and proteoglycans/glycoproteins, by mostly smooth muscle cells contributes to the conversion of fatty streaks into a mature atherosclerotic plaque [13]. Inflammatory cytokines, matrix metallo-proteinases, and tissue-factors which cause progression and consecutively potential destabilization of the protective fibrous cap are further released by activated macrophages [12]. Additionally, unstable atherosclerotic plaques were shown to be associated with leaky neovessels that penetrate the adventitia (neoangiogenesis) [15]. Arterial walls respond to atherosclerotic plaque growth by either eccentric growth in size (positive remodelling) [6] or decrease of luminal area (negative remodelling) [16]. Positive remodelling was shown to be associated with vulnerable plaques [17] and cannot reliably be detected by conventional luminographic imaging techniques. In the past, various studies in different patient collectives have shown that the primary characteristics of vulnerable plaques are high plaque

volume, large necrotic core, thin fibrous cap, positive vascular wall remodelling, and high density of neoangiogenesis [14, 18].

Specific Probes for Molecular Cardiovascular Magnetic Resonance Imaging

Specific or targeted probes for cardiovascular magnetic resonance imaging enable the in vivo visualization of cellular and molecular processes. Molecular MRI enables imaging with a substantially higher resolution compared to other molecular imaging modalities such as PET (positron emission tomography) and SPECT (single photon emission computed tomography). The drawback of molecular MRI compared to these imaging modalities however is, that higher in vivo concentrations of molecular probes and/or highly abundant molecular targets are needed for a reliable detection and quantification of the molecular probe in vivo.

Given that the signal intensity of MRI is based principally on the longitudinal (T1) and transverse (T2) relaxation time of free in vivo water protons, the functionality of the majority of MRcontrast agents and molecular probes is based on selective shortening of the T1 and T2 relaxation time. Visualization of specific receptors, molecules or cells is obtained by selective binding of the molecular MR probe. Another possibility to increase contrast enhancement is the accumulation of the probe on grounds of pathological tissue characteristics or specific cell types, e.g., the phagocytosis of iron oxide particles by macrophages [19].

T1-Shortening Molecular MR Probes

The most common administered T1 relaxation time shortening agents are gadolinium-based MR probes, which are based on chelate complexes with trivalent gadolinium at the center [20]. The T1 shortening effect of these probes is stronger compared to their T2 shortening effect. The benefit of Gdbased probes is the positive (T1) contrast generated on MRI images, which can be detected more easily, compared to the negative contrast generated by iron oxide particles. To improve in vivo sensitivity, new T1 shortening Gd-based MR probes are developed through conjugation of a specific vector (e.g., small molecules, peptides, or antibodies) with a Gd-chelate. These probes comprise Gd-containing liposomes [21], lipoproteins [22], and micelles [23]. By increasing the quantity of Gd-chelates per molecular probe and by binding of the molecular probe to its target, a more intense T1 effect of the probe can be induced [24].

T2-Shortening Molecular MR Probes

Molecular MR probes which shorten the local T2/T2 star relaxation time are primarily based on iron oxide particles. The shortening of the T2 relaxation time leads to a focal hypointense signal on T2/T2 star-weighted MR images. The T2-shortening effect of these probes is greater than its T1 effect, compared to Gd-based contrast agents [25]. Iron oxide particles can also be detected with higher sensitivity compared to Gd-based MR probes due to a more intense effect on the relaxation time [26]. T2 shortening molecular MR probes are commonly classified by their overall size, since their biological distribution is dependent on their size: ranging from monocrystalline iron oxide nanoparticles (MIONs,

<3 nm) to microsized iron oxide particles (MPIO, <10 µm). In the clinical setting, SPIOs, coated with dextran or carboxydextran, can also be used for liver and spleen imaging. They can be applied intravenously and are rapidly cleared from the blood by phagocytosis of cells of the reticuloendothelial system (RES) [27, 28], which results in a short blood half-life. USPIOs are characterized by long blood half-life as they bypass the RES due to their small size [27]. In clinical practice, these particles find common usage in imaging of atherosclerosis [29].

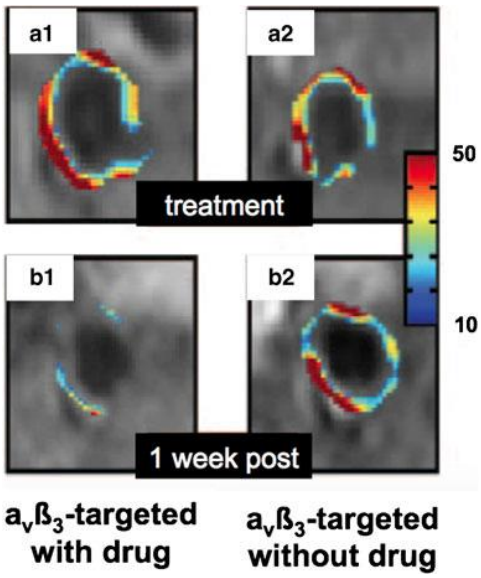

Fig. 1 Imaging of angiogenesis following the administration of Gdbased avß3-targeted nanoparticles in an animal model. The probe was combined with drug delivery of fumagillin and without fumagillin (A1, B1 and A2, B2). The signal intensity is given as percent-signalenhancement during treatment (A1, A2) and 1 week following treatment (B1, B2). (Adapted with permission from [32])

Molecular Vessel Wall Imaging

Various different markers exist for molecular imaging of atherosclerotic plaques. In this review article, we will introduce the most promising markers for molecular imaging of different pathological processes in cardiovascular disease.

One process that can be imaged is angiogenesis by the direct visualization of blood flow in the adventitia or by the visualization of specific marker proteins. The increased blood flow associated with angiogenesis can be assessed directly by applying unspecific MR contrast agents, which remain in the vasculature for a limited period of time. The signal from these contrast agents can be directly measured using a technique called DCE (dynamic contrast-enhanced) MRI [30]. The marker proteins which are specific for neovascularization are expressed on the surface of endothelial cells and include αvβ3 integrin [31]. Successful imaging of angiogenesis has been performed in an animal model (Fig. 1) using αvβ3 targeting liposomes containing gadolinium [32].

A further process which can be imaged using molecular MR probes is vascular inflammation. This process is associated with the influx of proinflammatory cells, e.g. monocytes/ macrophages, into the vascular wall. Different imaging approaches can be used to visualize macrophages. In recent years, most effort was put into the visualization of macrophages using iron oxide particles with different coatings. It is thought that iron oxide particles are taken up by monocytes within the vascular lumen. Subsequently, these monocytes migrate into the plaque and differentiate into macrophages. As this type of cell is not directly targeted by the iron oxide particles using a specific receptor or targeting protein this mechanism

is called passive targeting [33–36]. This type of particle cannot only be used to detect and quantify inflammation but also to assess response to therapy. One limitation of this approach is, however, that iron oxide particles lead to a negative signal, which can be more difficult to detect. Several different techniques have recently been proposed to overcome this limitation [37, 38]. These imaging approaches have been tested and validated in animal models and humans. A different approach to target macrophages is based on micelles targeted at a surface receptor of macrophages, called the scavenger receptor. This approach has, however, only been investigated so far in animal models [23]. A different group specifically targeted the scavenger receptor AI using a specific peptide on the surface of USPIOs and demonstrated a significantly increased atherosclerotic plaque accumulation compared to nontargeted USPIOs [39••].

A further interesting molecular target for the assessment of atherosclerosis is the extracellular matrix. Extracellular matrix proteins are expressed throughout the development of atherosclerotic plaques. The extracellular matrix is composed of different mainly structural proteins, e.g., different types of collagen or elastin as well as proteoglycans/glycoproteins. All of these proteins are highly abundant and represent one of the main overall components of the atherosclerotic plaque. Therefore, they represent an excellent target for molecular magnetic resonance imaging. One molecular probe which allows the unspecific targeting of extracellular matrix components is gadofluorine [40–42]. This agent, however, does not only visualizematrix components but is also associated with the lipid core of atherosclerotic plaques. Gadofluorine has so far only been tested in animal models. Two small molecular weight gadolinium-based specific MR probes have been used to visualize specific matrix associated plaque components. One is an elastin specific molecular probe. By visualizing the extracellular matrix with a high in vivo signal, this probe allows the assessment of the overall plaque burden as well as the density of elastic fibers within the atherosclerotic plaque matrix [38, 43••]. This molecular probe has so far been evaluated in small and large animal models and has potential to be translated into clinical studies (Fig. 2). A different also highly specific molecular probe is a fibrin specific probe. Fibrin in the matrix of the plaque plays an important role during the development of atherosclerotic lesions [44–47]. Additionally, fibrin is highly expressed on the surface of the plaque following plaque rupture. This gadolinium-based molecular probe is one of the very few molecular MR probes which have been successfully translated into a clinical setting. Using this agent, thrombus formation could be successfully imaged in patients. Taking a different imaging approach, HDL nanoparticles were functionalized with a collagen specific peptide and used to target intraplaque collagen during atherosclerotic plaque progression [48••].

Discussion and Conclusion

Our traditional view on atherosclerotic vessel wall disease has changed substantially due to a grown insight into its pathogenesis. Current conventional assessment of atherosclerosis is based on the angiographic evaluation of the degree of vascular stenosis. Various studies have demonstrated that the degree of stenosis is, however, not the most reliable parameter for the assessment of plaque vulnerability and its associated thrombotic vascular complications. Molecular MRI is based on imaging probes aiming at specific targets that play a crucial role in early and late atherosclerotic plaque formation. This may allow the clinical identification of vulnerable atherosclerotic plaques. There is a need for a shift from the angiographic/ anatomical assessment of plaques towards an additional molecular and cellular characterization of atherosclerotic plaques. This might lead to improved risk

stratification and to the identification of patients with vulnerable atherosclerotic plaque who are at high risk of complications from plaque rupture and who need urgent intervention.

Due to its high spatial resolution, excellent soft tissue contrast and ability to detect and quantify molecular probes, molecular MRI may be clinically employed for the above discussed purposes in the near future.

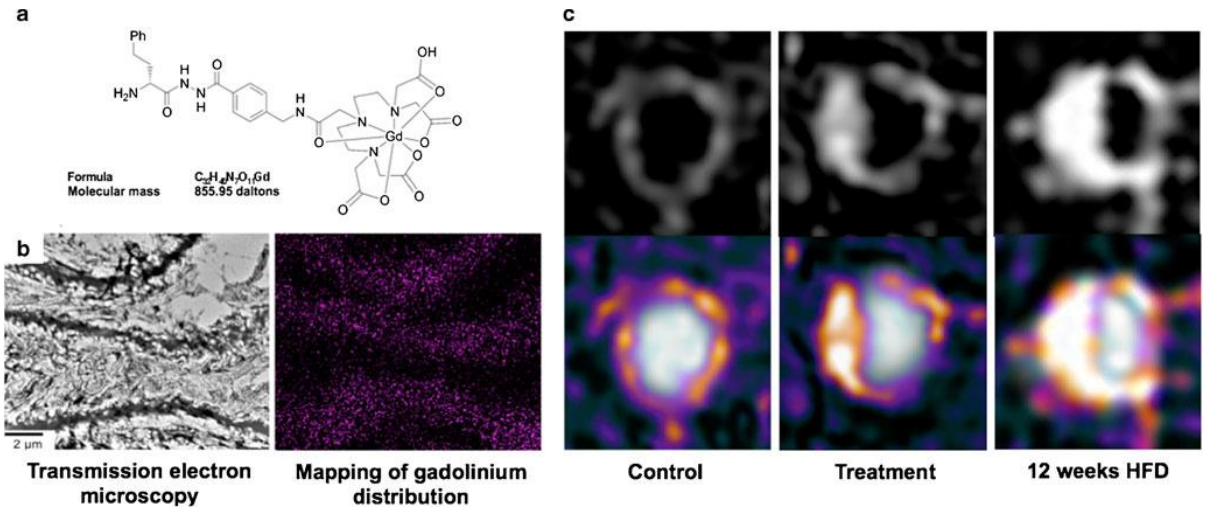

Fig. 2 Example on a gadolinium-based elastin-binding molecular probe for atherosclerotic plaque imaging in an animal model. a Structure of the elastin-binding molecular probe. b Gd distribution in the vessel wall sample (right) using TEM (transmission-electron-microscopy) (left). c Contrast-enhanced images (upper row) and angiogram (lower row) showing a significant increase in plaque burden in comparison to the control group. Statins resulted in a decrease in plaque burden. (Adapted from [43••].)

Compliance with Ethical Standards

Conflict of Interest

Yvonne Y. Bender, Hans U. Ebersberger, Andreas Pfeifer, Gerd Diederichs, Peter Hoppe, René M. Botnar, and Marcus R. Makowski declare that they have no conflict of interest. Bernd Hamm has received grant money from the following organizations to the Department of Radiology, Charité (Berlin, Germany): Abbott, Actelion Pharmaceuticals, Bayer Schering Pharma, Bayer Vital, BRACCO Group, Bristol-Meyers Squibb, Charité Research Organisation GmBH, Deutsche Krebshilfe, Dt. Stiftung für Herzforschung, Essex Pharma, EU Programmes, Fibrex Medical Ins., Focused Ultrasound Surgery Foundation, Fraunhofer Gesellschaft, Guerbetm, INC Research, InSightec Ltd., IPSEN Pharma, Kendel/ MorphoSys AG, Lilly GmBH, Lundbeck GmbH, MeVis Medical Solutions AG, Nexus Oncology, Novartis, Parexel CRO Service, Perceptive, Pfizer GmbH, Philipps, Sanofis-Aventis S.A., Siemens, Spectranetics GmbH, Terumo Medical

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Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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